TMTpro-18plex: The Expanded and Complete Set of TMTpro Reagents for Sample Multiplexing

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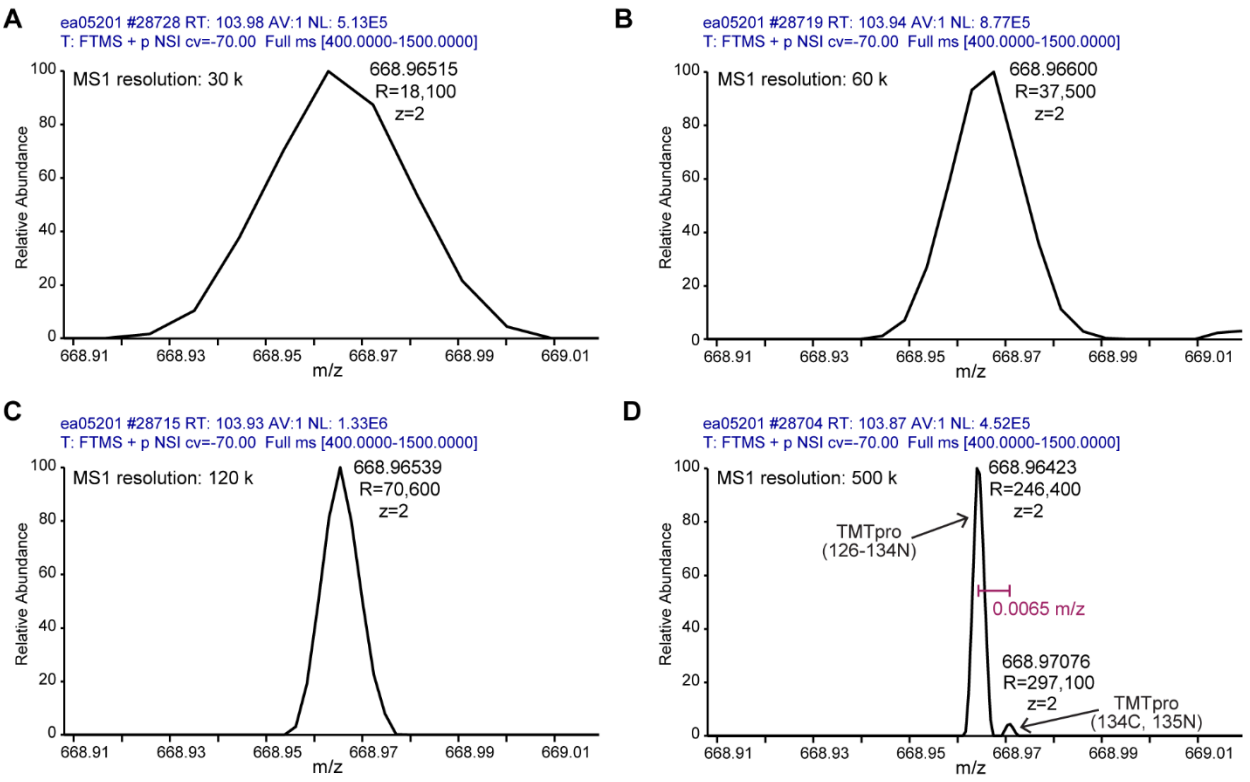

Figure S1. MS1 ion peaks for a TMTpro-18plex reagent-labeled precursor scanned with different MS1 resolutions in profile mode. The spectra originated from the 18-plex experiment in **Figure 2A** and consisted of 18 samples each labeled with a TMTpro-18plex reagent. The TMTpro-134C and TMTpro-135N reagent-labeled precursor (sequence: ALVILAK) has a +12 mDa monoisotopic mass shift (compared to the TMTpro16-labeled precursor) due to a difference in the number of ^{15}N and ^{13}C isotopes (there are two TMTpro reagents attached to this peptide, so +12 mDa mass difference in total). The precursor is doubly charged. Commonly used MS1 resolution settings (30,000, 60,000, and 120,000) are unable to resolve the 0.006 m/z mass difference (**A-C**). The MS1 resolution setting of 500,000 can detect the mass difference (**D**). The 0.0065 m/z difference in **(D)** is the measured m/z difference for the doubly-labeled and doubly-charged peptide. The theoretical m/z difference is 0.0063 m/z.

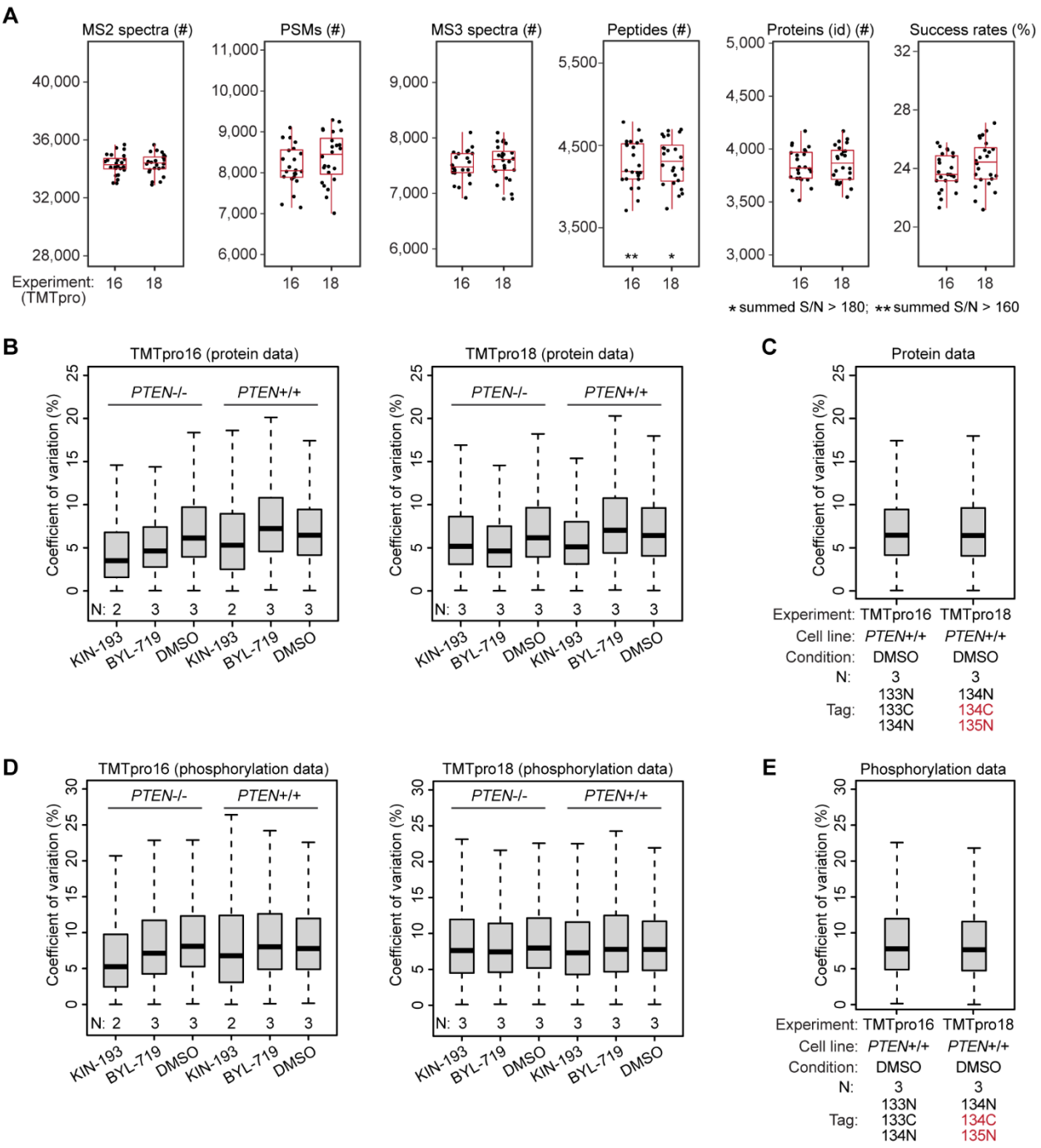

Figure S2. Evaluation of figures of merit in TMTpro16 and TMTpro18 experiments. (A) The TMTpro16 and TMTpro18 experiments showed comparable numbers of MS2 spectra, peptide-spectrum matches (PSMs), MS3 spectra, peptides, identified proteins, and success rates in each fraction. Each dot represents one of twenty-four fractions. **(B)** Coefficient of variation

(CV) distributions for the protein data in both experiments. A median CV of ~6% was achieved in both experiments. **(C)** Protein CV distributions focusing on the DMSO-treated control MCF10A (*PTEN*+/+) cell line, which include the samples labeled with the TMTpro-134C and TMTpro-135N reagents in the TMTpro18 experiment. **(D)** CV distributions for the phosphorylation data in both experiments. A median CV of ~7.5% was achieved in both experiments. **(E)** Phosphorylation CV distributions focusing on the DMSO-treated control MCF10A (*PTEN*+/+) cell line, which include the samples labeled with the TMTpro-134C and TMTpro-135N reagents in the TMTpro18 experiment.

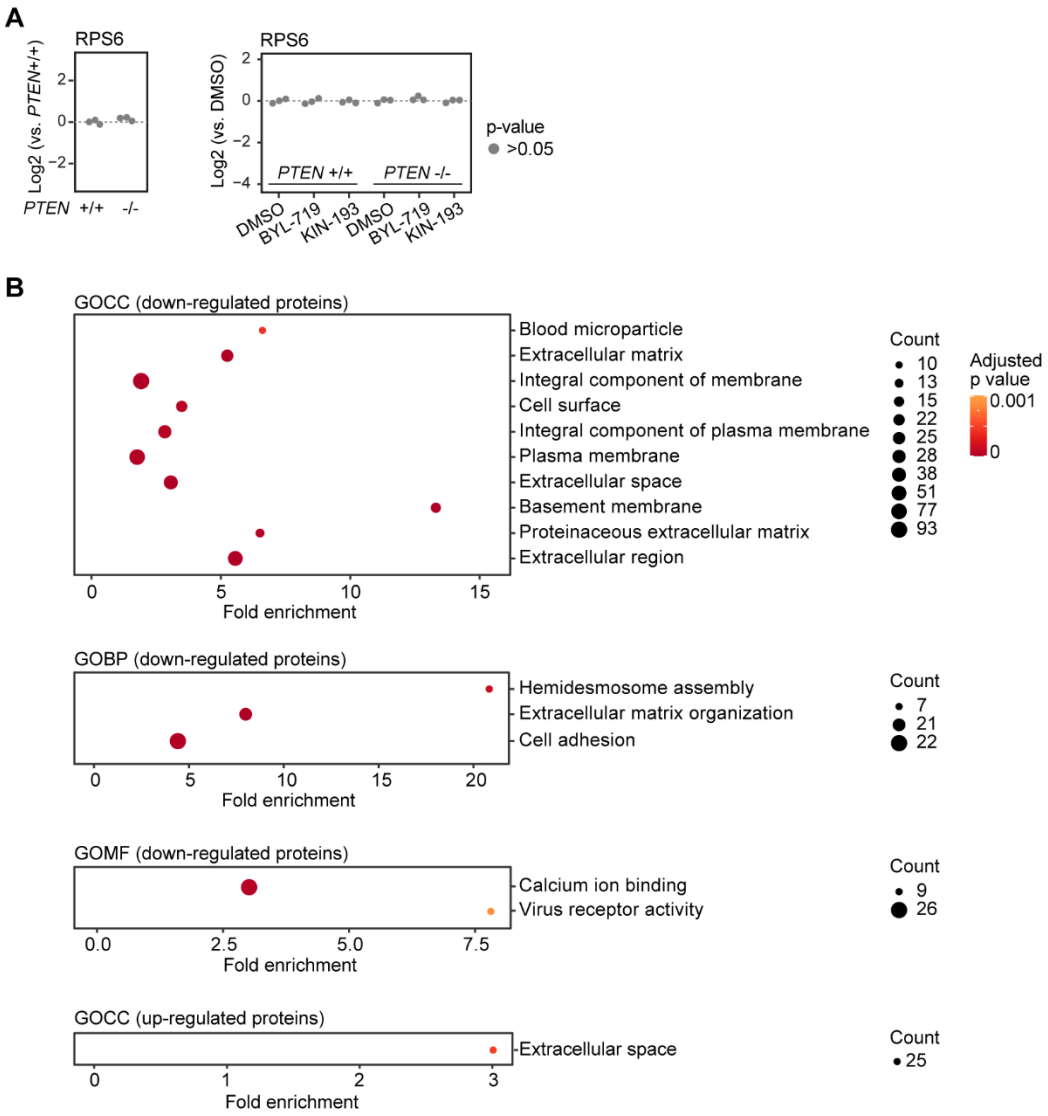

Figure S3. RPS6 protein abundance and gene ontology enrichment. (A) The RPS6 protein abundance was equivalent between control MCF10A and MCF10A *PTEN*-/- cell lines. The expression level of RPS6 remained unchanged after BYL-719 or KIN-193 treatments in both cell lines. **(B)** Gene ontology enrichment analysis of dysregulated proteins in MCF10A *PTEN*-/- cells (versus control MCF10A cells).

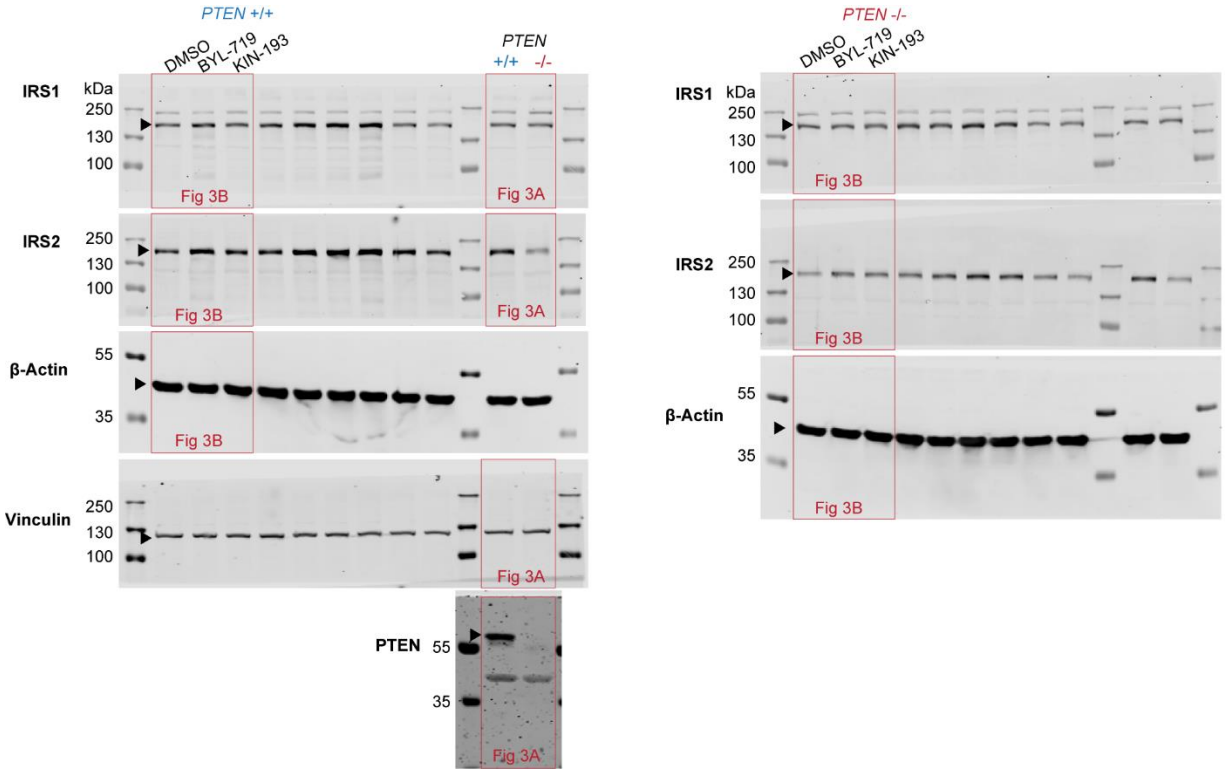

Figure S4. Raw Western blotting images.